Journal of Pharmacological Sciences 130 (2016) 110–116

HOSTED BY

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Combination therapy with renin-angiotensin-aldosterone system inhibitor telmisartan and serine protease inhibitor camostat mesilate provides further renoprotection in a rat chronic kidney disease model

Yuki Narita [a], Miki Ueda [b], Kohei Uchimura [c], Yutaka Kakizoe [b], Yoshikazu Miyasato [b], Teruhiko Mizumoto [b], Jun Morinaga [b], Manabu Hayata [b], Terumasa Nakagawa [b], Masataka Adachi [b], Taku Miyoshi [b], Yoshiki Sakai [d], Daisuke Kadowaki [a], Sumio Hirata [a], Masashi Mukoyama [b], Kenichiro Kitamura [c, *]

[a] Center for Clinical Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto, 862-0973, Japan
[b] Department of Nephrology, Kumamoto University Graduate School of Medical Sciences, 1-1-1 Honjo, Chuo-ku, Kumamoto, 860-8556, Japan
[c] Department of Internal Medicine III, Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Chuo-shi, Yamanashi, 409-3898, Japan
[d] Research Headquarters, Ono Pharmaceutical Co., Ltd., 1-8-2 Kyutaromachi, Chuo-ku, Osaka, 541-8564, Japan

ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form
24 December 2015
Accepted 7 January 2016
Available online 20 January 2016

Keywords:
Chronic kidney diseases
Serine protease inhibitor
Camostat mesilate
Telmisartan
Combination therapy

ABSTRACT

We previously reported that camostat mesilate (CM) had renoprotective and antihypertensive effects in rat CKD models. In this study, we examined if CM has a distinct renoprotective effect from telmisartan (TE), a renin-angiotensin-aldosterone system (RAS) inhibitor, on the progression of CKD. We evaluated the effect of CM (400 mg/kg/day) and/or TE (10 mg/kg/day) on renal function, oxidative stress, renal fibrosis, and RAS components in the adenine-induced rat CKD model following 5-weeks treatment period. The combination therapy with CM and TE significantly decreased the adenine-induced increase in serum creatinine levels compared with each monotherapy, although all treatment groups showed similar reduction in blood pressure. Similarly, adenine-induced elevation in oxidative stress markers and renal fibrosis markers were significantly reduced by the combination therapy relative to each monotherapy. Furthermore, the effect of the combination therapy on plasma renin activity (PRA) and plasma aldosterone concentration (PAC) was similar to that of TE monotherapy, and CM had no effect on both PRA and PAC, suggesting that CM has a distinct pharmacological property from RAS inhibition. Our findings indicate that CM could be a candidate drug for an add-on therapy for CKD patients who had been treated with RAS inhibitors.

1. Introduction

Hypertension and proteinuria have been recognized as two major risk factors for the progression of CKD. Conversely, the efficacy of antihypertensive drugs having renoprotective effect by the suppression of proteinuria, especially inhibitors of the renin-angiotensin-aldosterone system (RAS) has been proven in a number of clinical trials (1–3), and RAS inhibitors are becoming one of the few therapeutic drugs to prevent the progression of CKD. However, RAS inhibitors can only partially prevent the progression of CKD, and patients still show some progression of renal failure (4–6). To take full advantage of RAS inhibitors and fully prevent the progression of CKD, the development of an additional treatment is urgently needed.

In Japan, camostat mesilate (CM), an orally active synthetic serine protease inhibitor, has been clinically used for the treatment of chronic pancreatitis and postgastrectomy reflux esophagitis, and its clinical safety has already been established. CM has been reported not only to inhibit various serine proteases, but also to reduce proteinuria in both experimental and clinical glomerulonephritis (7–9). We previously demonstrated that CM had a renoprotective effect due to its natriuretic effect through the

* Corresponding author. Third Department of Internal Medicine, University of Yamanashi, 1110 Shimokato, Chuo-shi, Yamanashi, 409-3898, Japan. Tel.: +81 55 273 9602; fax: +81 55 273 9685.
E-mail address: kkenichiro@yamanashi.ac.jp (K. Kitamura).
Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2016.01.003

inhibition of a serine protease prostasin, a potent activator of epithelial sodium channel in the kidney (9). Subsequent studies from our laboratory clearly showed the renoprotective effects of CM through the suppression of proteinuria, oxidative stress, inflammation, and renal fibrosis independently of its blood pressure-lowering effect (10). In particular, CM attenuated oxidative stress by decreasing the expression of NADPH oxidase components and reduced renal fibrosis by inhibiting TGF-β_1 signaling (10, 11). Furthermore, we recently reported that CM has a potent direct radical scavenging activity independently of its inhibitory effect against serine proteases (12). These findings suggest the possibility that serine protease(s) is/are involved in the progression of CKD.

Although the renoprotective effect of CM has been shown by the previous studies described above, the effect of CM on RAS components has not been studied before. Moreover, it has not been investigated whether CM can provide an additional renoprotection on RAS inhibitors in the progression of CKD. Therefore, in the current studies, we examined if the renoprotective effect of CM depends on the RAS signaling and if CM could provide an additional benefit when combined with RAS inhibitors in a rat CKD model.

2. Materials and methods

2.1. Materials

CM was a kind gift from Ono Pharmaceutical Co., Ltd. (Osaka, Japan). TE was purchased as Micardis® Tablet from Astellas Pharma Inc. (Tokyo, Japan). Hydralazine (HYD) was purchased from Sigma–Aldrich (St. Louis, Mo., USA). Adenine and methylcellulose 400 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of the highest grade available from commercial sources.

2.2. Administration of CM and/or TE on the adenine-induced rat CKD model

All animal procedures were in accordance with the guidelines for care and use of laboratory animals approved by Kumamoto University. Thirteen-week-old male Sprague-Dawley rats (Japan SLC, Inc., Shizuoka, Japan) were used in this study. All rats were housed under controlled humidity, temperature, a 12:12-h light-dark cycle, and direct access to standard chow and tap water.

The adenine-induced rat CKD model was prepared following the established method (13). After having confirmed that serum creatinine levels were increased to approximately 4 mg/dL, the rats were divided into 6 groups; 1) control group (n = 8), 2) CKD group (adenine-induced CKD, n = 8), 3) TE group (adenine-induced CKD+TE, n = 8), 4) CM group (adenine-induced CKD+CM, n = 8), 5) CM+TE group (adenine-induced CKD+CM and TE, n = 6) and 6) HYD group (adenine-induced CKD+HYD, n = 6). CM (400 mg/kg/day) was suspended in distilled water and administered twice a day via oral gavage as previously described (12). Similarly, TE (10 mg/kg/day) was suspended in 0.5% methylcellulose 400 and administered twice a day via oral gavage. The doses of CM and TE were determined to have maximum renoprotective effect on rat remnant kidney model based on previous reports (12, 14). Because we found that the combination therapy with CM and TE showed greater reduction in SBP than each monotherapy in preliminary experiments, we arranged a CKD group which was treated by HYD to a similar SBP level with CM+TE. In our current study, HYD was administered via drinking water at a dose of 10 mg/kg/day. At the end of the study period, all rats were weighed, and systolic blood pressure (SBP) was measured by the tail-cuff method using a BP-98E manometer (Softron Co., Ltd., Tokyo, Japan). Twenty-four hour urine collections were made in metabolic cages and volume, electrolytes, and creatinine were measured. After a 5-week treatment period, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg) and blood samples and kidneys were collected. Creatinine and electrolytes in the blood were measured by a commercial laboratory (SRL, Tokyo, Japan) and kidneys were weighed and sliced into thin sections.

2.3. Histological studies

Resected kidneys were fixed with Dubosq-Brazil Fixative and embedded in paraffin. Embedded Kidney samples were sectioned at thicknesses of 2-μm and stained with periodic acid-Schiff (PAS) and Azan-Mallory. Azan-Mallory stained sections were photographed, and ten pictures were selected at random for histological analysis. The extent of interstitial fibrosis was estimated from Azan-Mallory stained sections. The fibrotic area was measured as the percentage of blue collagen staining in the tubulointerstitium with the exception of the tubular lumens and vessel walls using the Hybrid Cell Count image analysis (Keyence All-in-One Microscope BZ-X700, Osaka, Japan). The reactive oxygen species (ROS) production in the kidney was evaluated by dihydroethidium (DHE) staining as previously described (10), and images were captured by an Olympus BX50 with BH2-RFL-T3 (Olympus, Tokyo, Japan). Quantification of fluorescence intensity was determined by using Image-J (National Institutes of Health, Bethesda, MD, USA).

2.4. Real-time polymerase chain reaction (PCR)

Whole kidney total RNA was extracted with SV Total RNA Isolation Kit (Promega, Madison, MI), and 1 μg of total RNA was transcribed with Prime Script RT Master Mix kit (Takara Bio, Otsu, Japan). TaqMan probe for α-smooth muscle actin (α-SMA), plasminogen activator inhibitor-1 (PAI-1), and connective tissue growth factor (CTGF) and 28S ribosomal RNA were purchased from Applied Biosystems (Foster City, CA, USA). Real-time PCR was performed with a Light Cycler 480 (Roche Applied Science, Indianapolis, IN, USA). Statistical analysis of results was performed with the ΔCt (threshold cycle) value ($Ct_{\text{gene of interest}} - Ct_{\text{28S ribosomal RNA}}$). Relative gene expression was obtained from the ΔΔCt method ($Ct_{\text{sample}} - Ct_{\text{calibrator}}$).

2.5. Advanced oxidative protein products (AOPPs)

Plasma levels of AOPPs were determined by a protocol based on the method of Witko-Sarsat et al. (15). AOPPs concentrations were expressed in micromoles per liter of chloramine-T equivalents.

2.6. 2 − thiobarbituric acid reactive substances (TBARS)

Plasma levels of TBARS were measured by using a TBARS assay kit (JaICA, Shizuoka, Japan). TBARS concentrations were expressed in micromoles per liter of malondialdehyde (MDA) equivalents.

2.7. Immunoblot analysis

Pieces of kidney cortex were homogenized in ice-cold signal detection buffer. Aliquots of homogenized sample containing 30 μg of proteins were subjected to SDS-PAGE and transferred onto a polyvinylidene fluoride membrane. After being blocked with 5% skimmed milk powder in TBS-T, blots were probed with monoclonal antibodies against α-SMA (1:1,000, Dako, Glostrup, Denmark), fibronectin (1:10,000, Sigma–Aldrich, St. Louis, MO), and GAPDH (1:1,000, Cell Signaling Technology, Danvers, MA).

2.8. Statistical analysis

Results are expressed as means ± SE. Comparisons were made using ANOVA followed by the Newman-Keuls method for multiple comparisons. P values < 0.05 were considered as statistically significant. All statistical analyses were performed with the aid of GraphPad PRISM (GraphPad Software, La Jolla, CA).

3. Results

3.1. Effect of CM and/or TE treatment on general parameters

After 3 weeks of adenine administration, all five groups developed CKD as assessed by serum creatinine levels. The five CKD groups showed a decrease in body weight and creatinine clearance, and an increase in urinary volume and urinary sodium/potassium excretion ratio when compared with the control group, but there were no other statistically significant differences (Table 1). Serum creatinine levels were markedly elevated in the five CKD groups compared to the control group, and treatment groups except for the HYD group had decreased serum creatinine levels. Importantly, combination therapy with CM and TE resulted in a greater reduction in the serum creatinine levels compared with each monotherapy. Adenine administration markedly increased kidney weight in the five CKD groups, whereas treatment groups except for the HYD group demonstrated a significant decrease relative to the CKD group. Adenine-induced increase in serum potassium levels were significantly reduced in both CM group and CM+TE group, and no significant difference was observed between the two groups.

3.2. Effect of CM and/or TE treatment on systolic blood pressure

Fig. 1 shows the change in SBP at the end of the study period. No significant differences were observed in SBP between the control group and the CKD group. All treatment groups reduced SBP levels. The combination therapy group and the HYD group showed a further reduction in SBP to a similar extent, but there were no statistically significant differences between the combination therapy and each monotherapy.

3.3. Effect of CM and/or TE treatment on renal morphology

Kidneys from the five CKD groups showed marked renal swelling, discoloration, and deformity as compared with those from the control group. Treatment groups except for the HYD group attenuated gross morphologic injury, while combination therapy further ameliorated morphologic injury beyond the effect of each monotherapy (Fig. 2).

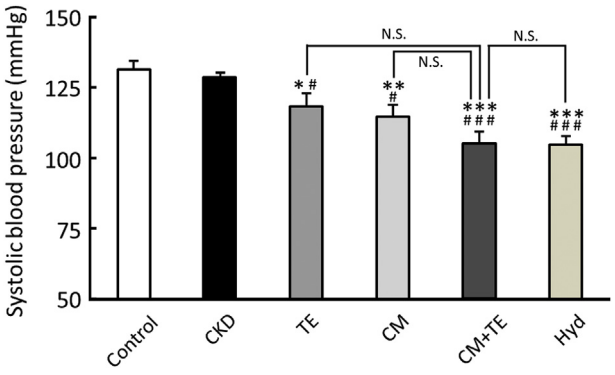

Fig. 1. Effect of CM and/or TE treatment on systolic blood pressure. At the end of the study period, systolic blood pressure (SBP) was measured by the tail-cuff method. Results are expressed as means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the Control group; $\#P < 0.05$, $\#\#\#P < 0.001$ compared with the CKD group.

3.4. Effect of CM and/or TE treatment on renal histology

Changes in renal histology were determined by PAS staining and Azan-Mallory staining of kidney sections. On PAS staining, no significant differences were observed in glomeruli among the six groups (Fig. 3A), but severe interstitial fibrosis with crystalline deposits were observed in the five CKD groups (Fig. 3B). In addition, on Azan-Mallory staining, each monotherapy reduced the severity of renal fibrosis evoked by adenine administration, and the combination therapy showed a greater decrease in renal fibrosis than each monotherapy (Fig. 3C, D).

3.5. Effect of CM and/or TE treatment on oxidative stress markers in the blood and kidney

We examined oxidative stress as a well-known mediator of kidney disease to clarify the mechanism by which combination therapy with CM and TE exerts its renoprotective effect. Plasma AOPP, a marker of protein oxidation, was increased in the CKD groups, while treatment groups except for the HYD group, had significantly suppressed plasma AOPP (Fig. 4A). Interestingly, combination therapy markedly reduced plasma AOPP beyond that of each monotherapy. Similarly, an increase in plasma TBARS, which is a major lipid peroxide marker, by adenine

Table 1
Data at the end of the experiment.

	Control	CKD	TE	CM	CM+TE	HYD
n	8	8	8	8	6	6
BW (g)	500 ± 6	376 ± 20***	388 ± 13***	333 ± 19***	350 ± 15***	360 ± 15***
KW/BW (mg/g)	11.1 ± 0.4	83.2 ± 7.7***	52.2 ± 4.3$^{***,\#\#}$	59.1 ± 8.2$^{***,\#}$	40.2 ± 3.7$^{***,\#\#\#,\S}$	72.5 ± 10.2***
UV (mL/day)	22 ± 1.3	80 ± 4.8***	81 ± 3.8***	72 ± 3.9***	70 ± 5.7***	80 ± 5.4***
Cre (mg/dL)	0.26 ± 0.01	2.01 ± 0.12***	1.55 ± 0.03$^{***,\#\#}$	1.51 ± 0.11$^{***,\#\#}$	1.23 ± 0.08$^{***,\#\#\#,\dagger,\ddagger,\S\S\S}$	1.81 ± 0.12***
Ccr (mL/min/kg)	7.61 ± 0.51	0.69 ± 0.1***	1.26 ± 0.06***	1.37 ± 0.14***	1.46 ± 0.18***	0.92 ± 0.14***
Na (meq/L)	144 ± 1	143 ± 1	142 ± 1	141 ± 1	140 ± 1	143 ± 2
K (meq/L)	3.9 ± 0.1	6.0 ± 0.1***,‡‡	5.6 ± 0.2***,‡	4.6 ± 0.4	5.1 ± 0.3**	5.7 ± 0.3***,‡
U-Na/K	0.48 ± 0.02	0.58 ± 0.01*	0.57 ± 0.02*	0.60 ± 0.03**	0.58 ± 0.03*	0.56 ± 0.01*

Results are expressed as means ± SE. CKD: chronic kidney disease, TE: telmisartan, CM: camostat mesilate, HYD: hydralazine, BW: body weight, KW/BW: kidney weight/body weight, UV: urine volume, Cre: serum creatinine, Ccr: creatinine clearance, Na: serum sodium, K: serum potassium, and U-Na/K: urinary sodium-to-potassium ratio. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with Control group; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ compared with CKD group; $\dagger P < 0.05$ compared with TE group; $\ddagger P < 0.05$, $\ddagger\ddagger P < 0.01$ compared with CM group; $\S P < 0.05$, $\S\S\S P < 0.001$ compared with HYD group.

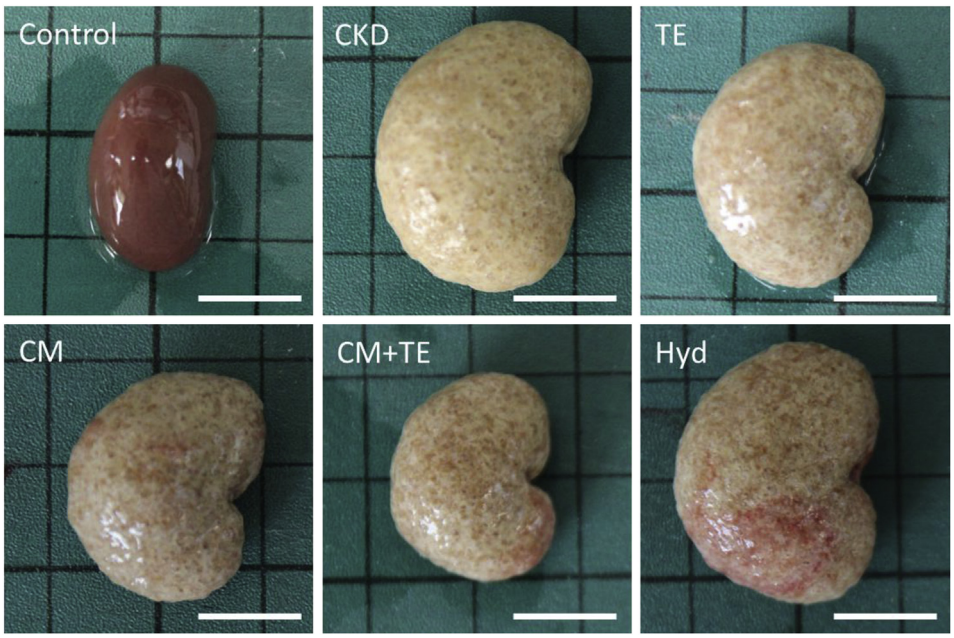

Fig. 2. Macroscopic morphology of kidneys from the six groups. Rat kidneys from the CKD groups show swelling, discoloration, and deformity as compared with those of the control group. The gross morphologic injury was attenuated by CM and TE respectively, but treatment with CM and TE further improved this condition. Scale bar: 10 mm.

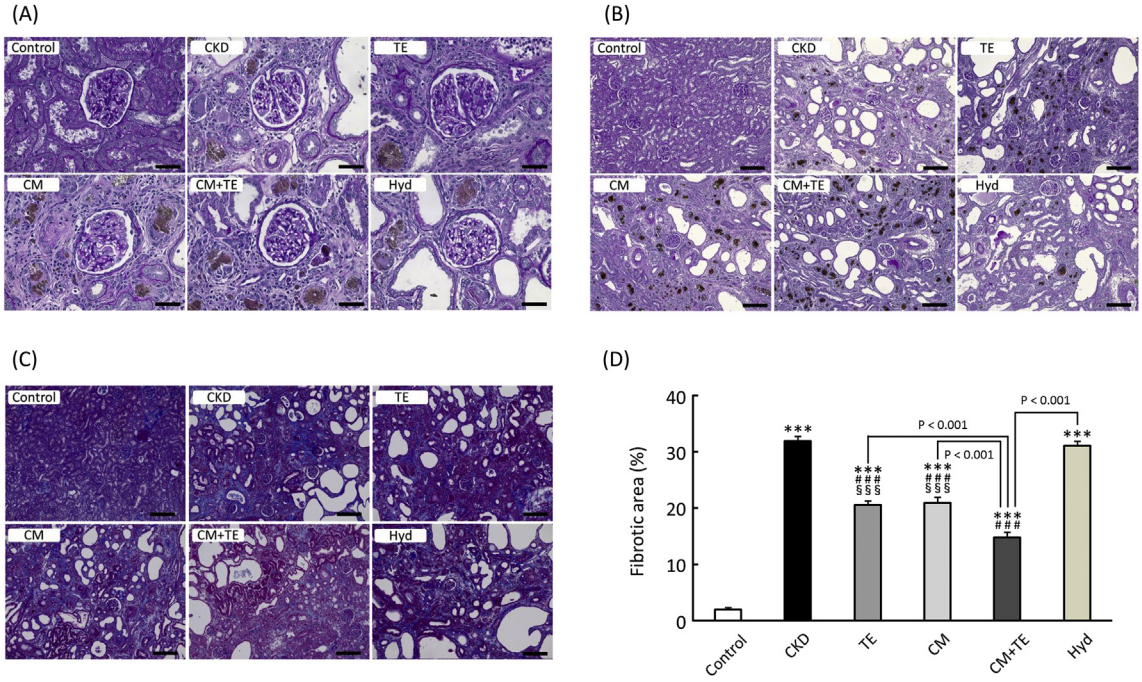

Fig. 3. Kidney histopathology. (A) Representative photomicrographs of PAS stained kidney sections. There were no marked differences among the six groups with respect to their glomeruli (×200). Scale bars: 50 μm. (B) Tubulointerstitial injury was significant in the CKD groups relative to the control group (×100). Scale bars: 200 μm. (C) Representative photomicrographs of Azan-Mallory stained kidney sections (×100). Scale bars: 200 μm. (D) The interstitial fibrosis areas were evaluated as described in the Materials and methods section, and the values are summarized in the bar graphs. Results are expressed as means ± SE. ***$P < 0.001$ compared with the Control group; ###$P < 0.001$ compared with the CKD group; §§§$P < 0.001$ compared with the HYD group.

administration was significantly reduced in the combination therapy in comparison with each monotherapy (Fig. 4B). Furthermore, DHE staining revealed that the combination therapy significantly reduced the accumulation of ROS relative to each monotherapy in the tubulointerstitial compartment (Fig. 4C, D).

3.6. Effect of CM and/or TE treatment on mRNA expression and protein levels of fibrotic markers

Because oxidative stress plays an important role in the development and progression of renal fibrosis, we evaluated the effect of the combination therapy on renal fibrosis by assessing fibrotic

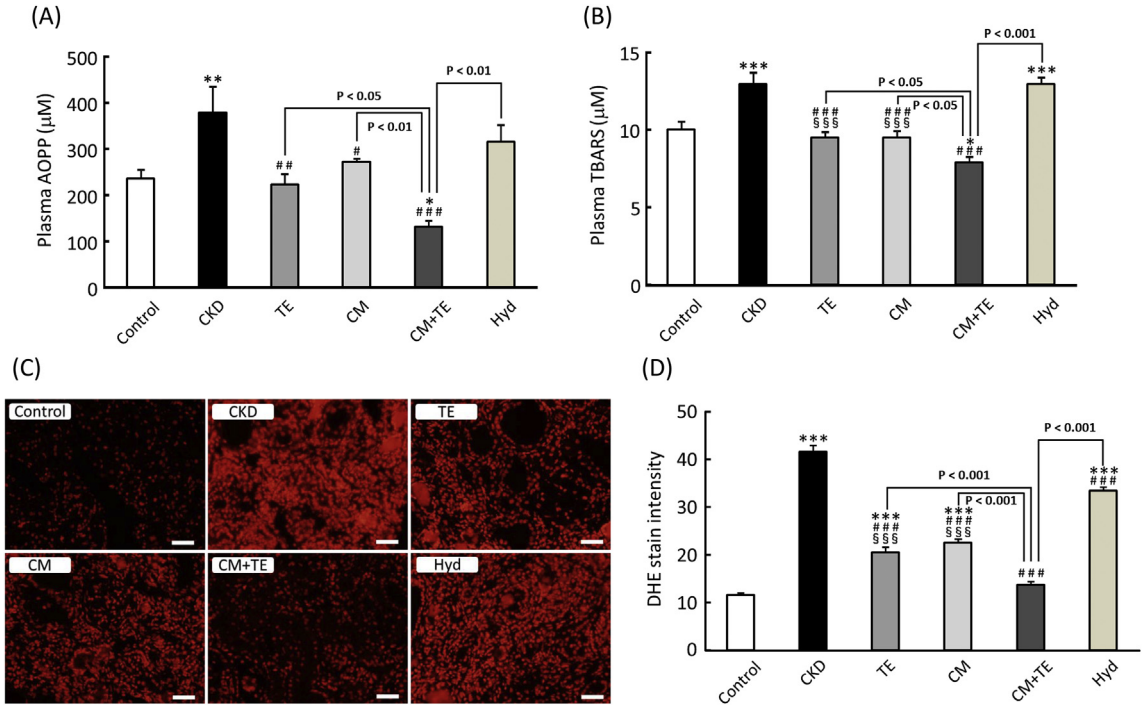

Fig. 4. Antioxidative effect of CM and/or TE treatment. (A) Advanced oxidative protein products (AOPPs) measurements. AOPPs concentrations were determined in the plasma by the method of Witko-Sarsat, and the values are summarized in the bar graph. (B) Plasma levels of 2-thiobarbituric acid reactive substances (TBARS) measurements. TBARS concentrations were measured by using a TBARS assay kit, and the values are summarized in the bar graph. (C) Dihydroethidium (DHE) staining. DHE staining of kidney sections (×100) is shown. Scale bars: 100 μm. Frozen sections were incubated with 0.1 mM DHE, and images were obtained with an Olympus BX50 with BH2-RFL-T3 (Olympus, Tokyo, Japan). (D) Quantification of fluorescence intensity was performed by using Image-J, and the values are summarized in the bar graph. Results are expressed as means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the Control group; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ compared with the CKD group; §§§$P < 0.001$ compared with the HYD group.

markers such as CTGF, α-SMA, PAI-1, and fibronectin. The mRNA expression of CTGF was markedly reduced in the combination therapy relative to each monotherapy (Fig. 5A). The mRNA expressions of α-SMA and PAI-1 showed a tendency to decrease in the combination therapy (Fig. 5B, C). Moreover, the protein levels of α-SMA and fibronectin were significantly decreased in the combination therapy compared to each monotherapy (Fig. 5D, E).

3.7. *Effect of CM and/or TE treatment on the RAS components*

We determined plasma aldosterone concentration (PAC) and plasma renin activity (PRA) to examine if CM modulates RAS components to exert its renoprotective effect. The monotherapy with TE and the combination therapy significantly reduced the adenine-induced elevation of PAC to a similar extent, while the monotherapy with CM or Hyd had no effect on it (Fig. 6A). Likewise, the monotherapy with TE and the combination therapy markedly ameliorated the adenine-induced suppression of PRA to a similar extent, while the monotherapy with CM and Hyd had no effect on it (Fig. 6B).

4. Discussion

The results in this study demonstrated the effect of a combination therapy with CM and TE on the progression of CKD in the adenine-induced rat CKD model. We found that the combination therapy provided further renoprotection relative to each monotherapy (Table 1). The combination therapy resulted in a greater reduction in oxidative stress and renal fibrosis compared to each monotherapy and the effect was independent of their blood pressure-lowering effect (Figs. 1, 4 and 5).

According to the ONTARGET study, the combination therapy with ramipril and telmisartan reduced urinary albumin excretion but failed to preserve renal function (16). On the other hand, Shiraishi et al. showed that the combination therapy with enalapril and nicorandil provided greater renoprotection in a rat CKD model (17). Namikoshi et al. showed that the combination therapy with candesartan and pioglitazone enhanced the renoprotective effect in Zucker obese rats (18). Therefore, it is reasonable to speculate that combination therapy can provide additional benefit if each medicine shows renoprotective effect through an independent mechanism. Our current study revealed that the combination therapy with CM and TE resulted in the greater renoprotection than each monotherapy. As shown in Fig. 6, the effect of the combination therapy on PAC and PRA was similar to that of TE monotherapy, and CM had no effect on both PAC and PRA. These findings suggest that the greater renoprotection in the combination therapy was attributed to a distinct pharmacological property of CM from RAS inhibition.

Although both CM and TE have been demonstrated to have an anti-oxidant activity by suppressing the NADPH oxidase component (19–21), the combination therapy with CM and TE showed further reduction in oxidative stress than each monotherapy in our current study. We speculated that this additive anti-oxidant effect of CM is partly due to its direct radical scavenging activity against hydroxyl radical (12). Previously, we demonstrated that CM inhibited the TGF-β_1 signaling in renal fibroblasts and subsequently suppressed the progression of renal fibrosis (11). RAS inhibiters have been shown to reduce the production of TGF-β_1 and subsequently suppress the progression of renal fibrosis (22, 23). Because CM and TE reduced renal fibrosis through a different mechanism, the combination therapy could result in a greater decrease in renal fibrosis than each monotherapy. In addition, since the oxidative stress is one of the

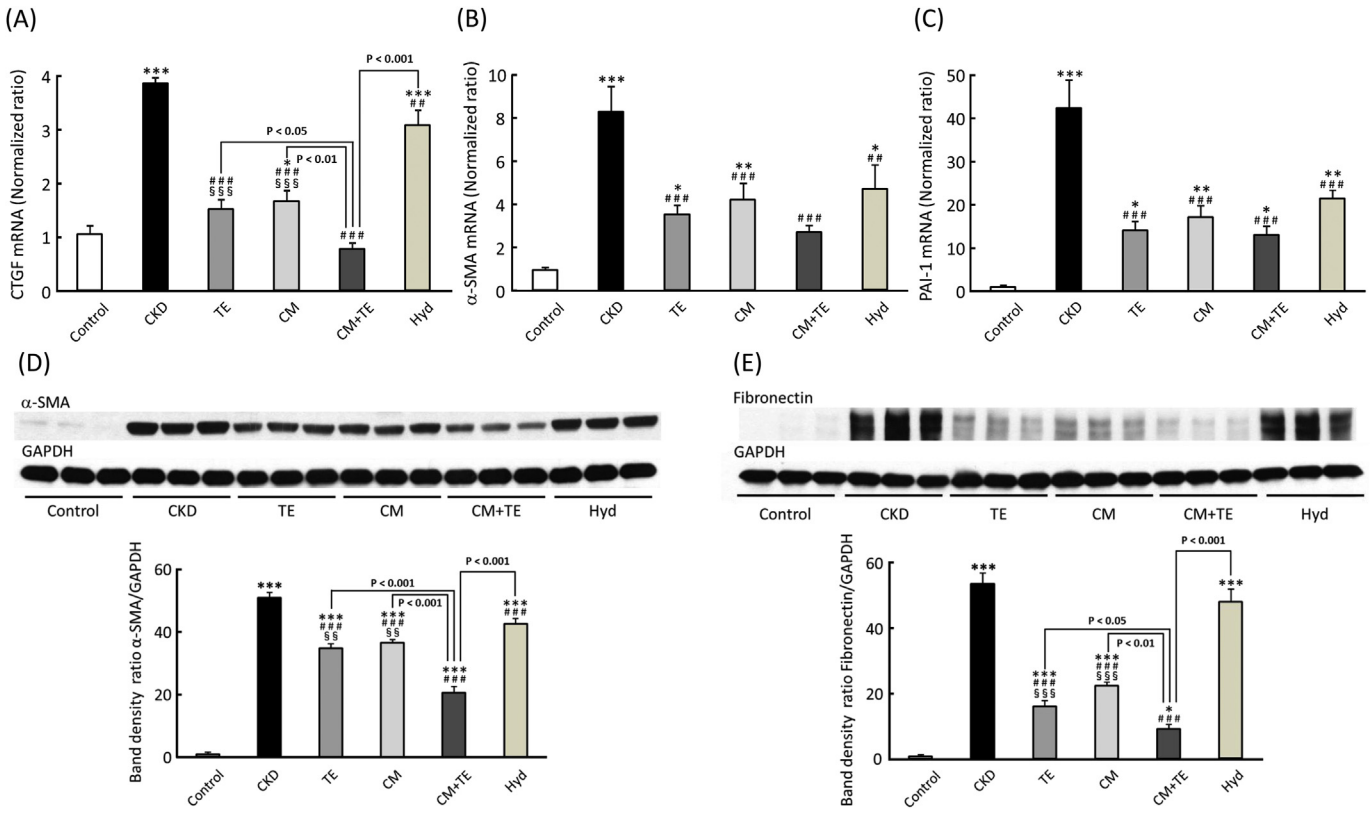

Fig. 5. Antifibrotic effect of CM and/or TE treatment. mRNA expressions of connective tissue growth factor (CTGF) (A), α-SMA (B), and plasminogen activator inhibitor-1 (PAI-1) (C) were determined by the real-time PCR. Expression of mRNA was normalized by 28S ribosomal RNA, and values are expressed as fold increase over the control group. Protein levels of α-smooth muscle actin (α-SMA) (D), fibronectin (E) and GAPDH were evaluated by immunoblot analysis. Densitometry values of α-SMA and fibronectin were normalized by GAPDH. Values are expressed as fold increases over the control group and shown in the bar graphs. Results are expressed as means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the Control group; $\#\#P < 0.01$, $\#\#\#P < 0.001$ compared with the CKD group; §§$P < 0.01$, §§§$P < 0.001$ compared with the HYD group.

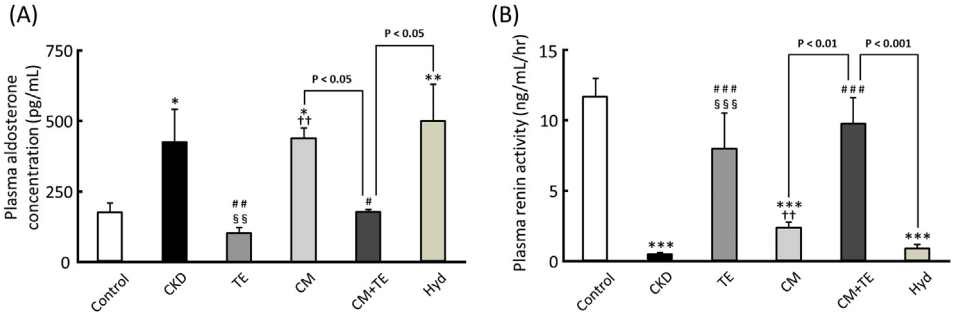

Fig. 6. Effect of CM and/or TE treatment on plasma aldosterone concentration and plasma renin activity. At the end of the study period, blood samples were collected from the abdominal aorta. Plasma aldosterone concentration (A) and plasma renin activity (B) were determined. Results are expressed as means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the Control group; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ compared with the CKD group; †$P < 0.05$, ††$P < 0.01$ compared with TE group; §§$P < 0.01$, §§§$P < 0.001$ compared with HYD group.

most important factors leading to renal fibrosis (24, 25), and the greater reduction in renal fibrosis in the combination therapy could be attributed to the further reduction in oxidative stress. In the current investigations, we were unable to determine the detailed mechanisms by which CM exerts its renoprotective effect. However, this is the first report showing the effect of combination therapy with CM and a RAS inhibitor on the progression of CKD.

In conclusion, the combination therapy with CM and TE provided further renoprotective effect than each monotherapy through the greater reduction in oxidative stress and renal fibrosis in an adenine-induced rat CKD model. Our present results suggest that CM could be a candidate drug for an add-on therapy for CKD patients who had been treated with RAS inhibitors.

Acknowledgment

The authors thank Ms. Noriko Nakagawa and Ms. Naoko Hirano (Graduate School of Medical Sciences, Kumamoto University) for their expertise in histopathology.

References

(1) Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med. 2001;345:851–860.
(2) Haller H, Ito S, Izzo JL, Januszewicz A, Katayama S, Menne J, et al. Olmesartan for the delay or prevention of microalbuminuria in type 2 diabetes. N Engl J Med. 2011;364:907–917.

(3) Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med. 2001;345:861–869.
(4) Fogari R, Zoppi A, Corradi L, Mugellini A, Lazzari P, Preti P, et al. Long-term effects of ramipril and nitrendipine on albuminuria in hypertensive patients with type II diabetes and impaired renal function. J Hum Hypertens. 1999;13:47–53.
(5) Estacio RO, Jeffers BW, Gifford N, Schrier RW. Effect of blood pressure control on diabetic microvascular complications in patients with hypertension and type 2 diabetes. Diabetes Care. 2000;23(Suppl. 2):B54–B64.
(6) Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. Heart Outcomes Prevention Evaluation Study Investigators. Lancet. 2000;355:253–259.
(7) Asami T, Tomisawa S, Uchiyama M. Effect of oral camostat mesilate on hematuria and/or proteinuria in children. Pediatr Nephrol. 2004;19:313–316.
(8) Ikeda Y, Ito H, Hashimoto K. Effect of camostat mesilate on urinary protein excretion in three patients with advanced diabetic nephropathy. J Diabetes Complicat. 1999;13:56–58.
(9) Maekawa A, Kakizoe Y, Miyoshi T, Wakida N, Ko T, Shiraishi N, et al. Camostat mesilate inhibits prostasin activity and reduces blood pressure and renal injury in salt-sensitive hypertension. J Hypertens. 2009;27:181–189.
(10) Hayata M, Kakizoe Y, Uchimura K, Morinaga J, Yamazoe R, Mizumoto T, et al. Effect of a serine protease inhibitor on the progression of chronic renal failure. Am J Physiol Ren Physiol. 2012;303:F1126–F1135.
(11) Morinaga J, Kakizoe Y, Miyoshi T, Onoue T, Ueda M, Mizumoto T, et al. The antifibrotic effect of a serine protease inhibitor in the kidney. Am J Physiol Ren Physiol. 2013;305:F173–F181.
(12) Ueda M, Uchimura K, Narita Y, Miyasato Y, Mizumoto T, Morinaga J, et al. The serine protease inhibitor camostat mesilate attenuates the progression of chronic kidney disease through its antioxidant effects. Nephron. 2015;129:223–232.
(13) Yokozawa T, Zheng PD, Oura H, Koizumi F. Animal model of adenine-induced chronic renal failure in rats. Nephron. 1986;44:230–234.
(14) Tsunenari I, Ohmura T, Seidler R, Chachin M, Hayashi T, Konomi A, et al. Renoprotective effects of telmisartan in the 5/6 nephrectomised rats. J Renin Angiotensin Aldosterone Syst. 2007;8:93–100.
(15) Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int. 1996;49:1304–1313.
(16) Mann JF, Schmieder RE, McQueen M, Dyal L, Schumacher H, Pogue J, et al. Renal outcomes with telmisartan, ramipril, or both, in people at high vascular risk (the ONTARGET study): a multicentre, randomised, double-blind, controlled trial. Lancet. 2008;372:547–553.
(17) Shiraishi T, Tamura Y, Taniguchi K, Higaki M, Ueda S, Shima T, et al. Combination of ACE inhibitor with nicorandil provides further protection in chronic kidney disease. Am J Physiol Ren Physiol. 2014;307:F1313–F1322.
(18) Namikoshi T, Tomita N, Satoh M, Haruna Y, Kobayashi S, Komai N, et al. Pioglitazone enhances the antihypertensive and renoprotective effects of candesartan in Zucker obese rats fed a high-protein diet. Hypertens Res. 2008;31:745–755.
(19) Sato-Horiguchi C, Ogawa D, Wada J, Tachibana H, Kodera R, Eguchi J, et al. Telmisartan attenuates diabetic nephropathy by suppressing oxidative stress in db/db mice. Nephron Exp Nephrol. 2012;121:e97–e108.
(20) Namikoshi T, Tomita N, Satoh M, Haruna Y, Kobayashi S, Komai N, et al. Olmesartan ameliorates renovascular injury and oxidative stress in Zucker obese rats enhanced by dietary protein. Am J Hypertens. 2007;20:1085–1091.
(21) Gounder VK, Arumugam S, Arozal W, Thandavarayan RA, Pitchaimani V, Harima M, et al. Olmesartan protects against oxidative stress possibly through the Nrf2 signaling pathway and inhibits inflammation in daunorubicin-induced nephrotoxicity in rats. Int Immunopharmacol. 2014;18:282–289.
(22) Lim DS, Lutucuta S, Bachireddy P, Youker K, Evans A, Entman M, et al. Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. Circulation. 2001;103:789–791.
(23) Lavoie P, Robitaille G, Agharazii M, Ledbetter S, Lebel M, Larivière R. Neutralization of transforming growth factor-beta attenuates hypertension and prevents renal injury in uremic rats. J Hypertens. 2005;23:1895–1903.
(24) Cho MH, Jung KJ, Jang HS, Kim JI, Park KM. Orchiectomy attenuates kidney fibrosis after ureteral obstruction by reduction of oxidative stress in mice. Am J Nephrol. 2012;35:7–16.
(25) Ozbek E, Ilbey YO, Ozbek M, Simsek A, Cekmen M, Somay A. Melatonin attenuates unilateral ureteral obstruction-induced renal injury by reducing oxidative stress, iNOS, MAPK, and NF-kB expression. J Endourol. 2009;23:1165–1173.